PATENT COOPERATION TREATY

PCT

REC'D 27 OCT 2005
WIPO PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 041407woJHcw	FOR FURTHER ACTION See Form PCT/IPEA/416	
International application No. PCT/EP2004/051234	International filing date *(day/month/year)* 24.06.2004	Priority date *(day/month/year)* 24.06.2003
International Patent Classification (IPC) or national classification and IPC C12M1/113, C12M1/16, C12N1/14, C12R1/645		
Applicant H\FER BIOREACT GMBH		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 11 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 7 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
30.07.2005	25.10.2005
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Gruber, M Telephone No. +31 70 340-9824

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/051234

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-38	as originally filed

Claims, Numbers

1-29	received on 06.09.2005 with letter of 02.09.2005

Drawings, Sheets

1/18-18/18	as originally filed

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/051234

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 1-15(partially),17-22(partially)

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 1-15(partially),17-22(partially)

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☒ See separate sheet for further details

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/051234

Box No. IV Lack of unity of invention

1. ☐ In response to the invitation to restrict or pay additional fees, the applicant has:
   - ☐ restricted the claims.
   - ☐ paid additional fees.
   - ☐ paid additional fees under protest.
   - ☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	17,21,22,28
	No:	Claims	1-16,18-20,23-27,29
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-29
Industrial applicability (IA)	Yes:	Claims	1-29
	No:	Claims	

2. Citations and explanations (Rule 70.7):

   see separate sheet

Re Item III.

Present claims 1 to 15 and 17 to 22 relate to an extremely large number of possible methods (claims 1 to 15,17,and 19 to 22) and products (claim 18) because these claims all refer to "microorganisms" or an "enzyme and/or metabolite mixture" which is produced by these "microorganisms". Support within the meaning of Article 6 PCT within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the methods and products claimed. In the present case, the claims so lack support, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search and the examination have been carried out for those parts of the claims which appear to be supported, namely those parts relating to the methods and products which use the microorganisms mentioned in the four examples of the description. They correspond to those mentioned in claim 5 (ii).

For deciding on this limitation, only those claims have been taken into consideration for which an International Search Report and a Written Opinion have been established.

Re Item IV.

Specification according to Rule 40.1 PCT of the reasons for which the International application PCT/EP04/51234 is not considered as complying with the requirements of invention according to Rule 13 PCT.

Reference is made to the following document:

D1: GUTIERREZ-CORREA MARCEL ET AL: "Mixed culture solid substrate fermentation of Trichoderma reesei with Aspergillus niger on sugar cane bagasse" BIORESOURC TECHNOL; BIORESOURCE TECHNOLOGY MAY 1999 ELSEVIER SCI LTD, EXETER, ENGL, vol. 68, no. 2, May 1999 (1999-05), pages 173-178, XP001204371

D5: EP-A-0 140 723 (MULTIBIO SA) 8 May 1985 (1985-05-08)

1 According to the Administrative Instructions und der the PCT (in force from 1 July

1998), annex B, part 1(c), unity of inventions has to be considered in the first place only relation to the independent claims:

The application contains five (5) independent claims:

Claim 1 relates to a culture method for producing a defined enzyme mixture and/or metabolite mixture
Claim 18 relates to an enzyme mixture and a metabolite mixture obtainable according to the method of any one of claims 1 to 17
Claim 19 relates to a fermentation method for processing one or more target substrates with an enzyme mixture and/or metabolite mixture obtainable by the method of any one of claims 1 to 17
Claim 20 relates to a method for the cultivation of microorganisms at equal growth rates
Claim 23 relates to a bioreactor

1.1 There is no common technical feature present between the five independent claims.

A common technical features between the independent claims 1,18, and 19 is an enzyme mixture and/or metabolite mixture. This feature, however, is known in the art, e.g. from D1, ref. item V of this Report.

A common technical feature between the independent claims 20 and 22 is the parameter "water activity". This parameter which is understood as being equivalent to the parameter "moisture content". The latter, however, is known in the art, ref. D1, page 174, paragraph 2.3 with the title "Solid substrate fermentation".

A common technical feature between the independent claims 19 and 23 is a fermentation step of a substrate. This however, is known in the art, e.g. from D1.

A common feature of claims 1 and 23 is a reactor in which solid-phase bioreactions can take place. Such a reactor, however, is known from D5 (see also

under item V, Second invention, of this report).

No "common" or "corresponding" special (new and inventive) technical feature is present between the six independent claims, as required by Rule 13.2 PCT.

1.2 Furthermore, there is no common problem present between the six independent claims which could serve as the single general inventive concept as required by Rule 13.1 PCT.

Independent claims 1 and 18 refer to the problem of how to cultivate a defined enzyme and/or metabolite mixture.
Independent claim 19 refers to the problem of how to run a fermentation with a defined enzyme and/or metabolite mixture.
Independent claim 20 refers to the problem of how to cultivate microorganisms at equal growth rates.
Independent claim 23 refers to the problem of how to construct a reactor for cultivation of a defined enzyme and/or metabolite mixture.

1.3 Consequently, there is no unity present between the five independent claims, as required by Rule 13 PCT.

2 The application is split into three (3) groups of (alleged) inventions:

Group 1: claim 1 (and its dependent claims 2 to 15,17) and claims 18,19
Group 2: claim 20 and its dependent claims 21 to 22
Group 3: claim 23 and its dependent claims 24 to 29

2.1 No "common" or "corresponding" special (new and inventive) technical feature is present between the three groups of (alleged) inventions, as required by Rule 13.2 PCT.

2.2 Furthermore, no common problem could be recognised between the three groups

which could serve as a single general inventive concept as required by Rule 13.1 PCT.

Consequently, there is no unity present between the three groups, as required by Rule 13 PCT.

3 For the Preliminary Examination Report, the subject matter of Group 2 has been treated together with the subject matter of Group 1.

Re Item V.

The following documents are referred to in this communication:

D1 : GUTIERREZ-CORREA MARCEL ET AL: "Mixed culture solid substrate fermentation of Trichoderma reesei with Aspergillus niger on sugar cane bagasse" BIORESOURC TECHNOL; BIORESOURCE TECHNOLOGY MAY 1999 ELSEVIER SCI LTD, EXETER, ENGL, vol. 68, no. 2, May 1999 (1999-05), pages 173-178, XP001204371

D2 : MADAMWAR D ET AL: "FORMATION OF CELLULASES BY CO-CULTURING OF TRICHODERMA-REESEI AND ASPERGILLUS-NIGER ON CELLULOSIC WASTE" WORLD JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY, vol. 8, no. 2, 1992, pages 183-186, XP009041572 ISSN: 0959-3993

D5: EP-A-0 140 723 (MULTIBIO SA) 8 May 1985 (1985-05-08)

First Invention (claims 1-15,17-19,22-24)

1 The present application does not meet the requirements of Art. 6 PCT because the subject matter of claims 2,13,14, and 15 is not clear.

The mentioned claims relate to a very high number of individual methods because they all comprise various alternatives which are linked by "and/or". Therefore, it

appears impossible to acknowledge novelty and inventive step (Art. 33(1) PCT) for these claims.
By contrast, claims 3,7 to 12 and 17 which also comprise various alternatives, relate to process parameters and possible uses of an obtained product (mixed enzyme and/or metabolite mixture) which are merely considered as examples and not inventive concepts if taken individually.

2 The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1,3 to 12,18,19 and 20 is not new in the sense of Article 33(2) PCT.

2.1 Document D1 discloses a method for producing a defined enzyme mixture and/or metabolite mixture comprising contacting *Aspergillus niger* and *Trichoderma reesei* with a solid substrate (sugar cane bagasse) and carrying out fermentation under specific ("selective") conditions as outlined in paragraph 2.3 on page 174. The inoculum was obtained in a preculture (paragraph 2.2.). As it is set out in the introduction on page 173, the main objective is to convert ligniocellulose biomass into an energy saving form such as ethanol. The ligniocellulose biomass to be used can be provided by the agricultual sector and one principal species for this is sugar cane bagasse. Therefore, ligniocellulose biomass can be considered as a "target substrate" according to the definition of the present applicantion. Furthermore, soymeal, urea or ammonium sulphate can be considered as "inducer substrates".
As the microorganisms used in D1 are identical to those of the present application (those which were searched and examined) and as no specific ("selective") culture conditions are mentioned in claim 1, it can be acknowledged that the obtained enzyme and/or metabolite mixture in D1 is suitable for use in the processes and areas mentioned in claims 7 to 12.

D1 is considered novelty destroying for the subject matter of claims 1,3 to 12, 18 and 19.

2.2 Document D2 discloses a method for producing a defined enzyme mixture (cellulases) comprising contacting *Aspergillus niger* and *Trichoderma reesei* with

the solid ("target") substrate lignocelluloses and carrying out fermentation under specific ("selective") conditions. Urea or ammonium sulphate can be considered as "inducer substrates". As a selective parameter, the water content of the substrate has been used and examined (ref. Tab. 2). The parameter "water content" is not identical to "water activity" (or moisture content) but another parameter to quantify the amount of water in relation to the (dry) weight of the substrate. Therefore, the concept in D1, i.e examining enzyme production as a function of the water quantity in the substrate, is considered equivalent to the subject matter of claim 20.

The inoculum was obtained in a preculture (paragraph "Materials and Methods"). As the microorganisms used in D2 are identical to those of the present application (those which were searched and examined) and as no specific ("selective") culture conditions are mentioned in claim 1, it can be acknowledged that the obtained enzyme and/or metabolite mixture in D2 is suitable for use in the processes and areas mentioned in claims 7 to 12.

Document D2 is considered novelty destroying for the subject matter of claims 1,3 to 12,18,19 and 20.

3 It is, at present, not clear to which extent the subject matter of claims 17,21 and 22 causes unexpected or surprising effects with respect to what is disclosed in the above mentioned prior art. Therefore, no inventive activity can be acknowledged for the time being (Art. 33(3) PCT).

Second Invention (claims 23-29)

1 The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 23 to 27 and 29 is not new in the sense of Article 33(2) PCT.

Document D5 discloses a bioreactor (ref. Fig. 1, claims) for fermentation of solid substrates comprising a fermentation module (4), feeding means (3) for feeding

the solid substrate into the fermentation module, an induction module (12) for adding reagents to the fermentation media, a harvesting module (18) comprising outlet means, and conveying means comprising a conveying screw located in the housing of the fermentation tank. The regulation means (11,12) comprise aeration (12) and liquid feeding means (11) which are connected to the housing wall.

Document D5 is considered novelty destroying for the subject matter of claims 23 to 27 and 29.

Remark:
An apparatus (such as a bioreactor) cannot (legally) be defined by formulations relating to its intended use such as "... for performing the culturing method ...". Therefore, such a formulation does not provide for any legal protection.

2 The subject matter of claim 28 is, although not disclosed in Document D5 - not considered to result in an unexpected or surprising, in other words non obvious effect with regard to the available prior art. Therefore, no inventive activity can be acknowledged for the subject matter of claim 28.

PCT/EP2004/051234 JH/cw

Höfer Bioreact GmbH September 2, 2005

Claims

1. A culture method for producing a defined enzyme mixture and/or metabolite mixture optimized for the fermentation of one or more target substrates by contacting an inoculating mixed culture of microorganisms in a solid-phase bioreactor with one or more target substrates or a combination of one or more target substrates and one or more inducer substrates, and by keeping the mixed culture under an appropriate selection pressure by a suitable selection of the culturing parameters and by a specific induction by the target and/or inducer substrate and/or inhibition with appropriate inhibitors for a defined culturing time.

2. The method according to claim 1, wherein the inoculating mixed culture step

(i) is obtainable by culturing a preculture of mixed microorganisms, preferably a mixed culture of fungi, adapted to solid or liquid, optimally inductive substrates cultivating on normal agar plates other "solid-state" (SSF) cultures, such as column reactors with inert carriers as a medium for supporting growth, or in any liquid cultures, such as shaking flasks or fermenter cultures as an inoculating culture for the subsequent main SSF cultures; and/or

(ii) is a culture which has run through an inductive preculture and one or more main cultures operated under selection pressure; and/or

(iii) is a culture which has run through an inductive preculture and one or more main cultures operated under selection pressure and is suitable to be employed for fermentation of the target substrate either directly or after preservation by freezing and/or lyophilization.

3. The method according to claim 1 or 2, wherein

(i) the appropriate selection pressure is built up and maintained by suitably selecting the culturing parameters, preferably selected from moisture content (water activity), pH value, temperature, oxygen availability, redox potential and nutrient composition; and/or

(ii) the inductive substrates and/or target substrates are selected from all kinds of raw or waste materials of natural (microbial, vegetable, animal or human) and non-natural industrial origin and their mixtures, preferably the inductive substrate is selected from any plant-, animal- or microbial material and the target substrate is selected from any plant-, animal- or microbial material which have to be modified or degraded.

4. The method of claim 3, wherein the water activity is used for controlling the selection pressure, preferably the water activity is hold lower than one, preferably between 0.85 and 0.99, by the addition of water and its removal by means of temperature and suction.

5. The method according to any one of claims 1 to 4, wherein at least two microorganisms are employed for producing mixed cultures and precultures of mixed microorganisms, preferably

(i) fungi (ascomycetes, deuteromycetes) of the genera *Penicillium* spec., *Aspergillus* spec., *Trichoderma* spec., *Fusarium* spec., *Eurotium* spec., *Absidia* spec., *Neurospora* spec., *Mucor* spec., *Chaetomium* sp., *Rhizopus* sp. etc. are employed as microorganisms; or

(ii) fungi (ascomycetes, deuteromycetes) of the species *Penicillium chrysogenum*, *Eurotium amstelodami*, *Aspergillus niger*, *Aspergillus tubingiensis*, *Trichoderma harzianum*, *Trichoderma atroviride*, *Trichoderma reesei*, *Fusarium oxysporum* and *Neurospora intermedia* are employed as microorganisms; or

(iii) fungi (white rot fungi, brown rot fungi) of the genera *Trametes* spec., *Pleurotus* spec., *Phanerochaete* spec., *Nematoloma* spec. and *Agaricus* spec. etc. are employed as microorganisms, most preferably fungi (white rot fungi) which produce laccase or manganese peroxidase, such as organisms of genera *Trametes* spec., *Pleurotus* spec., *Phanerochaete* spec. and *Agaricus* spec. etc., are employed as microorganisms; or

(iv) fungi (white rot fungi) such as organisms of genera *Trametes* spec., *Pleurotus* spec., *Phanerochaete* spec. and *Agaricus* spec. etc. which, in addition to lignolytic enzymes, such as laccase or manganese peroxidase, secrete further oxidases which produce H_2O_2, such as glucose oxidases I and II (GOD), glyoxal oxidase, methanol oxidase, galactose oxidase, cellobiose

quinone oxidoreductase (CBQ) or cellobiose dehydrogenase (CDH) etc., are employed as microorganisms; or

(v) bacteria (actinomycetes) of the genus *Streptomyces* spec. etc. are employed as microorganisms;

most preferably the H_2O_2 required for the peroxidase action is added by metering.

6. The method according to any one of claims 1 to 5 which is performed in a continuous manner or in a step-wise manner with one or more process cycles.

7. The method according to any one of claims 1 to 6, wherein the continuously produced enzyme/substrate/fungus mixtures

(i) suitable applied as such, or after separation of the substrate/fungus mixture to obtain a liquid enzyme cocktail; and/or

(ii) are suitable to be used for the saccharification of all kinds of natural polysaccharide substrates or for the degradation of vegetable, animal or microbial polymers; and/or

(iii) are substituted by enzymes which are prepared by means of other methods or which are commercially available; and/or

(iv) are suitable for fermentation under essentially anaerobic or anaerobic conditions.

8. The method according to any one of claims 1 to 7, wherein the mixed cultures are suitable for the continuous production of specific hydrolase cocktails and/or oxidoreductase cocktails for processes target substrates, preferably in the wood-processing industry, paper and pulp industries, textile industry, leather industry, animal-processing industry, detergent industry, fodder industry, food industry, waste water, exhaust air and soil purification, in the processing of residual materials and in the processing of raw materials from naturally renewable resources.

9. The method according to claim 1 or 8, wherein the enzyme mixture is a hydrolytic/oxidative enzyme cocktail and is suitable

(I) for the enzymatic extraction (hydrolytic saccharification) of sugar beet chips at least by means of a two-phase culture; or

(ii) for the enzymatic extraction (hydrolytic saccharification, polymer degradation) of, for example, chemically pre-extracted materials, such as sugar cane, cereals and other vegetable, animal or microbial raw or waste materials; or

(iii) for the enzymatic extraction (hydrolytic saccharification, polymer degradation) of vegetable, animal or microbial raw or waste materials before a chemical and/or enzymatic and/or microbial treatment, such as special fermentations, or after them.

10. The method according to any one of claims 1 to 9 for producing an enzyme mixture (hydrolytic enzyme cocktails/oxidative enzyme cocktails) suitable for the enzymatic extraction (hydrolytic saccharification) of sugar beet chips or other polysaccaride containing material, wherein

(i) the inducer substrate is a rape extraction material; and/or

(ii) the microorganisms are *A. niger* and *A. tubigensis*; and/or

(iii) the water activity is initially set to be about 0.99; and/or

(iv) during the culture process *Neurospora intermedia* is added to the mixed culture and the water activity is reduced to about 0.96.

11. The method according to any one of claims 1 to 9 for producing an enzyme mixture (hydrolytic enzyme cocktails/oxidative enzyme cocktails) suitable for the enzymatic extraction (hydrolytic saccharification) of grass silage or other polysaccaride containing material, wherein

(i) the inducer substrate is a rape extraction material; and/or

(ii) the microorganisms are *A. niger*, *A. tuglbensis* and *Neurospora intermedia*; and/or

(iii) the water activity is initially set to be about 0.98; and/or

(vi) during the culture process *Trichoderma atroviridae* and grass silage as substrate are added to the mixed culture and the water activity is raised to about 0.99.

12. The method according to any one of claims 1 to 9 for producing an enzyme mixture (hydrolytic enzyme cocktails/oxidative enzyme cocktails) suitable for the enzymatic extraction (hydrolytic saccharification) of corn silage or other polysaccaride containing material, wherein

(i) the inducer substrate is a rape extraction material; and/or

(ii) the microorganisms are *A. niger*, *A. tubigensis* and *Neurospora intermedia*; and/or

(iii) the water activity is initially set to be about 0.98; and/or

(iv) during the culture process *Aspergillus oryzae* and corn silage as substrate are added to the culture and the water activity is raised to about 0.99.

13. The culturing method according to any one of claims 1 to 12, wherein after optimum inoculation and selective process operation, the preinduced mixtures of microorganisms and enzyme mixtures (hydrolytic/oxidative enzyme cocktails)

(i) are either directly supplied to the downstream processes, such as special fermentations (methane fermentations), or first passed to a pre-hydrolysis container to effect a preliminary saccharification or, in the optimum case, a complete hydrolysis of the polysaccharides or other polymers, such as proteins and fats; or

(ii) are transferred to another solid state process operation in which the whole substrate which is to be fermented later is selectively utilized for producing enzymes and at least partially hydrolyzed.

14. The method according to claims 1 and 13, wherein

(i) said preinduced mixtures of microorganisms are mixtures of white rot fungi or mixtures of organisms which metabolize only low amounts of sugar at high enzyme forming rates; or

(ii) after optimum inoculation and selective process, the preinduced mixtures of microorganisms and enzyme mixtures (hydrolytic/oxidative enzyme cocktails), which were produced in a side stream in addition to the main solid-state process operation, are incorporated together with the main reaction into the subsequent fermentations by means of mixed populations of other microorganisms; or

(iii) after optimum inoculation and selective process operation according to the invention, the preinduced mixtures of microorganisms and enzyme mixtures (hydrolytic/oxidative enzyme cocktails) are transferred to another solid state process operation in which the whole substrate is selectively utilized for producing enzymes for composting purposes; or

(iv) after optimum inoculation and selective process operation according to the invention, the preinduced mixtures of microorganisms and enzyme mixtures (hydrolytic/oxidative enzyme cocktails) are transferred to another solid state process operation in which the whole substrate is selectively utilized for producing enzymes for the degradation of xenobiotics; or

(v) after optimum inoculation and selective process operation according to the invention, the preinduced mixtures of microorganisms and enzyme mixtures (hydrolytic/oxidative enzyme cocktails) are flowed through by liquid or gaseous induction substrates which are degraded or converted by the enzymes formed.

15. The method according to any one of claims 1 to 14, wherein the solid-phase cultures are generally performed in screw reactors, drum reactors, tower reactors, trickling film reactors, solid-state air-lift reactors, horizonatal mixers, vertical mixers etc. according to the principle of screw conveying, pressure screw conveying, conveying belt transport etc., optionally modified or in a cascade form, preferably the solid-phase cultures are performed

(i) in a screw reactor either singly or arranged in a cascade form; or

(ii) in special solid-state air-lift reactors; or

(iii) as batch cultures, fed-batch cultures or continuously.

16. The method according to any one of claims 1 to 15 which further comprises

conservation of the obtained mixed culture by decreasing the water activity during the fermentation process, preferably by air flow through the substrate or by a final drying step, preferably in a fluidised bed or belt dryer.

17. The method according to any one of claims 1 to 16, wherein a leaching of the produced enzyme containing solid (enzyme mixture) is carried out by moving or stirring it with water, buffer, detergent/water or detergent/buffer solutions, preferably in an amount of 1 to 10 or 1 to 20 by weight (enzyme containing solid to solution), for 30 min to 2hours and wherein the obtained enzyme slurry is filtered and the filtrate is further used as a solvent for additional leaching cycles (up to 10 times) for receiving a highly concentrated enzyme slurry.

18. An enzyme mixture and a metabolite mixture obtainable according to the method of any one of claims 1 to 17.

19. A fermentation method for processing one or more target substrates which comprises fermenting the target substrates with an enzyme mixture and/or metabolite mixture obtainable by the method of any one of claims 1 to 17.

20. A method for the cultivation of microorganisms at equal growth rates by adjusting the water activity.

21. The method according to claim 20, wherein the cultivation of several microorganisms is controlled by sequential modification of water activity during the fermentation of solid substrates, allowing to cultivate two microorganisms at the same growth rate.

22. The method according to claim 20 or 21, wherein the cultivation of several pairs of microorganisms is controlled by

(i) sequential decrease of water activity during the fermentation of solid substrates, allowing to cultivate several appropriate pairs of microorganisms at the same growth rate; or

(ii) sequential increase of water activity during the fermentation of solid substrates, allowing to cultivate several appropriate pairs of microorganisms at the same growth rate.

23. Bioreactor for performing the culturing method according to one of the claims 1 to 22 , comprising

a fermentation module (10) for the fermentation of substrates under selection pressure whereby the fermentation module (10) comprises regulation means (28, 32) to adjust a fermentation environment,

a feeding means (16) being connected to the fermentation module (10) to feed the substrate,

an induction module (12) for adding reagents (agents conferring selection pressure) to the fermentation media,

a harvesting module (14) comprising outlet means and

a conveying means (24) to convey the media from the fermentation module (10) through the induction module (12) to the harvesting module (14).

24. Bioreactor according to claim 23 whereby the conveying means (24) is located within a common housing (22).

25. Bioreactor according to claim 23 or 24 whereby the regulation means comprises aeration means (32) and/ liquid feeding means (28).

26. Bioreactor according to one of the claims 23–25 whereby the aeration means (32) and/ or the liquid feeding means (28) are connected to a housing wall.

27. Bioreactor according to one of the claims 23-26 whereby the conveying means (24) comprises a conveying screw.

28. Bioreactor according to one of the claims 23-27 whereby the aeration means (38) and/ or a liquid feeding means are connected to a hollow shaft (36) of the conveying screw (24).

29. Bioreactor according to one of the claims 23-28 whereby the induction module (12) comprises an aeration means (46) and/ or a liquid feeding means.